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# Vesicles of Mixtures of the Bolaform Amphiphile Sodium Di-*n*-decyl $\alpha,\omega$ -Eicosanyl Bisphosphate and Sodium Di-*n*-decyl Phosphate<sup>†</sup>

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Various properties of vesicles prepared from different ratios of the bolaform (bipolar) amphiphile disodium di-*n*-decyl  $\alpha,\omega$ -eicosanyl bisphosphate (EBP) and the nonbolaform (monopolar) sodium di-*n*-decyl phosphate (DDP) have been studied. The colloidal stability decreased upon the increasing sodium di-*n*-decyl phosphate content. The main phase transitions, studied by fluorescence depolarization and differential scanning calorimetry, become less cooperative and occur at decreasing temperatures upon increasing sodium di-*n*-decyl phosphate content. The bolaform amphiphile was found to be membrane spanning as indicated by the lamellar repeat spacing obtained by X-ray powder diffraction. Electron microscopic studies of the small vesicles prepared from pure EBP revealed that addition of the fusogen  $\text{Ca}^{2+}$  caused formation of tubules for the EBP. The tubules did not revert to vesicles upon addition of the  $\text{Ca}^{2+}$  chelator EDTA, whereas mixed vesicles with 20–50 mol % DDP content showed enlarged vesicles after subsequent addition of  $\text{Ca}^{2+}$  and EDTA. A preliminary conclusion is that a substantial amount of nonbolaform amphiphiles must be present in a bolaform membrane to enable fusion to occur.

## Introduction

The term bolaform amphiphile describes a molecule that consists of two hydrophilic headgroups connected by one or two long hydrophobic chains. Bolaform amphiphiles can form monolayer membranes instead of bilayer membranes. Structurally related to the bolaform amphiphiles are gemini amphiphiles. These are molecules consisting of two hydrophobic chains with their hydrophilic headgroups connected by a short spacer. Geminis usually form micellar aggregates in solution and have very low critical micelle concentrations and low Krafft points.<sup>1,2</sup>

In nature, bolaform amphiphiles occur in the membranes of archaeobacteria as isoprenoid glycerol ether lipids. Archaeobacteria are also called extremophiles, referring to the extreme circumstances under which they live, including low pH, high temperatures, and very high salt concentrations.<sup>3</sup> Much research has been done on vesicles formed from natural bolaform membrane lipids. The proton permeability of the monolayer membrane and the rate of contents release are very low compared to bilayer membranes.<sup>4–8</sup>

Recently, research has been focused on the synthesis of novel synthetic bolaform amphiphiles,<sup>9–14</sup> because of the many different morphologies they display in water,<sup>15</sup> e.g.,

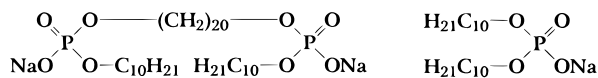
vesicles<sup>16–20</sup> and rods.<sup>21,22</sup> Generally vesicles are formed when the spacer between the headgroups is long or when the headgroups are connected by two spacers. Some bolaform amphiphiles have membrane-disrupting action.<sup>23,24</sup> Others can be used as ion channels in bilayer vesicles.<sup>25,26</sup>

We became interested in vesicles prepared from mixtures of synthetic bolaform amphiphiles and their nonbolaform counterpart, because the membrane lipids of archaeobacteria are not solely bolaform. Most archaeobacterial membranes consist of both bolaform and nonbo-

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**Figure 1.** Structures of disodium di-*n*-decyl  $\alpha,\omega$ -eicosanyl bisphosphate (EBP, left) and sodium di-*n*-decylphosphate (DDP, right).

laform lipids, the ratio depending on the species.<sup>3</sup> In those membranes the bolaform molecules are membrane spanning which influences the bilayer characteristics. The main phase transition ( $T_c$ ) is expected to occur at a higher temperature in bolaform-containing vesicles than the phase transition of a bilayer vesicle. E.g., the  $T_c$  of DDP is 35 °C<sup>27</sup> whereas the  $T_c$  of its bolaform counterpart sodium di-*n*-dodecyl  $\alpha,\omega$ -tetracosanyl bisphosphate is 60.5 °C.<sup>20</sup> In addition, bolaform lipids affect the fusion behavior. Relini et al.<sup>7,8</sup> found that in vesicles of archaeobacterial membrane lipids at least 5% nonbolaform lipids must be present to allow  $\text{Ca}^{2+}$  induced fusion.

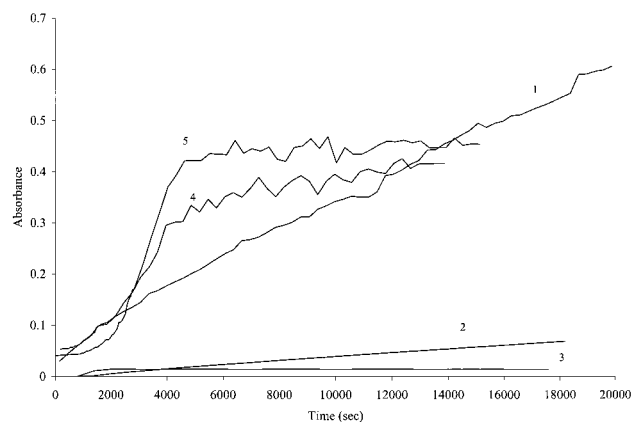
Recently, however, slow fusion of vesicles of solely bolaform archaeobacterial lipids has been observed.<sup>6</sup> This is contradictory to expectations based on current membrane fusion models. According to these models, the bilayers aggregate first, facilitated by a divalent cation in case of negatively charged amphiphiles. Subsequently a hemifusion intermediate is formed, followed by complete fusion.<sup>28,29</sup> According to these crucial steps in the fusion event, the leaflets of the bilayer have to act independently to allow fusion. This is not possible when bolaform lipids or amphiphiles form the membrane or monolayer, because the inner and outer leaflets of the bilayer are connected. Therefore vesicles of bolaform amphiphiles will aggregate upon  $\text{Ca}^{2+}$  addition but resist fusion.

It is likely that the formation of a fusion intermediate requires that one of the headgroups of the bolaform amphiphile molecule moves from the water phase through the apolar lipid phase to the other side of the membrane. The observation that the glycerol headgroup of archaeobacterial membrane lipids can move through the hydrocarbon phase can explain the fusion of vesicles of bolaform archaeobacterial lipids.<sup>7,8,30</sup> However, it is very unlikely that the charged headgroups of the bolaform EBP can diffuse through the hydrocarbon matrix. On the other hand, if the vesicles of this amphiphile contained some proportion of nonbolaform phosphate, then some degree of fusion might be expected to occur.

This report describes the properties of vesicles composed of different proportions of bolaform and nonbolaform amphiphiles. We prepared vesicles consisting of a bolaform amphiphile, di-*n*-decyl  $\alpha,\omega$ -eicosanyl bisphosphate (EBP), combined in various ratios with a nonbolaform amphiphile, sodium di-*n*-decyl phosphate (DDP). The amphiphile structural formulas are shown in Figure 1.

## Results and Discussion

**Colloidal Stability.** Vesicle solutions prepared from pure EBP in doubly distilled water remained clear for several weeks at ambient temperature. Upon an increase of the content of DDP, the stability decreased. Vesicles composed of EBP and DDP in a molar ratio of 4:1, respectively, were stable for several days. The vesicles of



**Figure 2.** Optical density vs time curves of vesicle preparations composed of various EBP/DDP mixtures: 1 = EBP; 2 = EBP/DDP (4:1); 3 = EBP/DDP (1:1); 4 = EBP/DDP (1:4); 5 = pure DDP.

EBP/DDP in molar ratios of 1:1 and 1:4 formed precipitates after several hours. Vesicles composed of pure DDP in doubly distilled water precipitated after less than 3 h. It has been observed before that vesicles of short di-*n*-alkyl phosphates precipitate readily at ambient temperature.<sup>27</sup>

The stability was also measured by monitoring the optical density (O.D.) at 400 nm of a vesicle solution in an aqueous 50 mM NaCl solution. The O.D. vs time curves are shown in Figure 2.

Here it becomes evident that in the presence of the electrolyte the stability of the vesicles is altered significantly. The electrostatic headgroup repulsion is reduced when vesicles are in a high ionic strength solution. The stability of vesicles of charged amphiphiles in a high salt solution is therefore lower than the stability in a low salt solution. Figure 2 shows that vesicles of EBP/DDP (1:1 and 4:1, molar ratios) were the most stable. Vesicles containing more DDP precipitate rapidly. In the presence of electrolytes the vesicles formed from pure EBP were also less stable than the vesicles formed from EBP/DDP in the molar ratios of 1:1 and 4:1. This observation may be explained as follows. In very small bilayer vesicles, the number of molecules in the outer leaflet is almost twice as large as the number of molecules in the inner leaflet. This asymmetric distribution is impossible in monolayer vesicles. As a result there are gaps between the outer headgroups in a highly curved monolayer of bolaforms.<sup>31</sup> When DDP is present, it can fill those gaps. This also allows a larger curvature of the membrane. We contend that the vesicles of EBP/DDP (molar ratios 1:1 and 4:1) can be smaller than those of pure EBP vesicles. This decreases the van der Waals attraction and slows down precipitation. The differences between the stability of the various vesicles in water and in the NaCl solution can therefore be rationalized: the role which vesicle size plays in stability is larger in high ionic strength media than in pure water, because of the decreased electrostatic repulsion in the high salt solution.

**Phase Behavior.** The phase transition temperatures ( $T_m$ ) for the different amphiphile mixtures were determined by fluorescence depolarization and differential scanning calorimetry (DSC). The results are summarized in Table 1.

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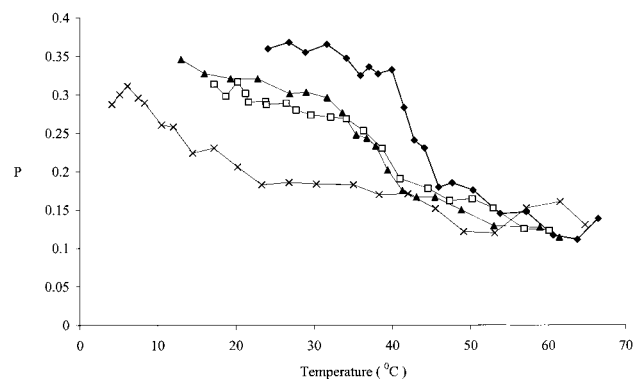
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**Table 1. Main Phase Transition Temperatures of Vesicles of Different EBP/DDP Ratio<sup>a</sup>**

sample	$T_m$ (°C) <sup>b</sup>	$T_m$ (°C) <sup>c</sup>	$\Delta_m H_{int}$ (kcal (mol monomer) <sup>-1</sup> )
1. EBP	43 (40–46) <sup>d</sup>	31.7	6.66
2. EBP/DDP (4:1)	41 (34–47) <sup>d</sup>	27.9	4.00
3. EBP/DDP (1:1)	38 (32–44) <sup>d</sup>	[35.9] <sup>f</sup>	7.72
4. EBP/DDP (1:4)	<34 <sup>e</sup>	[35.8] <sup>f</sup>	8.04
5. DDP	<20 <sup>e</sup>	[35.6] <sup>f</sup>	10.89

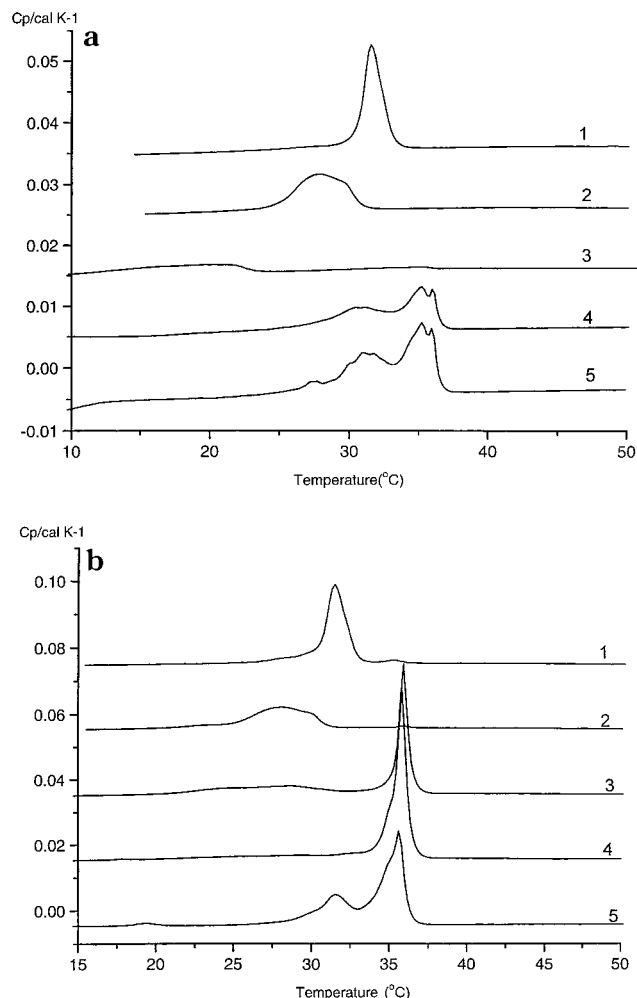
<sup>a</sup> All vesicles prepared by stirring the amphiphile solution in hot water ( $T > 50$  °C). <sup>b</sup> Determined by fluorescence depolarization. <sup>c</sup> Determined by differential scanning calorimetry. <sup>d</sup> Listed values are the midpoints of the transition range. <sup>e</sup> Scattering at low temperatures complicated the determination of the phase transition temperature. <sup>f</sup> See text.

**Figure 3.** Fluorescence depolarization experiments for EBP (◆), EBP/DDP 4:1 (□), EBP/DDP 1:1 (▲), EBP/DDP 1:4 (×), DDP (○).

The transitions from the gel phase to the liquid crystalline phase in the surfactant mixtures become less cooperative (broader) with increasing proportions of DDP, and  $T_m$  is decreased with increasing proportions of DDP as indicated by fluorescence depolarization measurements (Figure 3). The  $T_m$  decreases from 43 °C for vesicles formed from pure EBP to 41 °C for vesicles composed of EBP/DDP (molar ratio 4:1). The vesicles composed of EBP/DDP, molar ratio 1:1, have a  $T_m$  of 38 °C. When vesicles of pure DDP and of EBP/DDP, molar ratio 1:4, were measured at temperatures lower than ambient, the polarization value fluctuated (Figure 3). This was probably caused by scattering of the emission and excitation radiation by precipitating amphiphile molecules in accordance with the observations described above. It was therefore difficult to determine the  $T_m$  of the vesicles of these compositions.

DSC measurements were performed to determine more precise phase transition temperatures. Unlike fluorescence depolarization, no probe was used. The enthalpograms are shown in Figure 4. Vesicles of pure EBP have a  $T_m$  of 31.7 °C and the  $T_m$  of vesicles of EBP/DDP (4:1) is 27.9 °C. The enthalpograms of EBP/DDP (1:1 and 4:1) and pure DDP show very complicated phase behavior (Figure 4a). The enthalpograms obtained after equilibrating the samples of EBP/DDP (1:1 and 4:1) and pure DDP for 11 h below the phase transition temperature show sharp peaks at about 35 °C (Figure 4b).

We suggest that these peaks indicate the (re)solubilization of DDP that precipitated during the equilibration time. Previously it was found that at 35 °C myelin formation of DDP occurs.<sup>32</sup> Myelins are formed at the interface of water and surfactant. They are multilamellar

**Figure 4.** (a) DSC curves after 0 h of equilibration at low temperature: 1 = EBP; 2 = EBP/DDP 4:1; 3 = EBP/DDP 1:1; 4 = EBP/DDP 1:4; 5 = DDP. (b) DSC curves 11 h after equilibration at low temperature: 1 = EBP; 2 = EBP/DDP 4:1; 3 = EBP/DDP 1:1; 4 = EBP/DDP 1:4; 5 = DDP.

wormlike structures in which stacked bilayers, alternating with water layers, are concentrically wrapped around a core-axis of water.<sup>33</sup> Myelin formation is accompanied by an endothermic transition, and  $T_{myelin}$  has also been defined as a crystal-to-liquid-crystal phase transition temperature.<sup>34</sup> At this temperature a sudden increase in solubility occurs, comparable to the Krafft temperature for micellar systems. Vesicles of EBP/DDP (1:1 and 1:4) and pure DDP are not stable at temperatures below  $T_{myelin}$ (DDP). Below  $T_{myelin}$ (DDP) the vesicles revert to insoluble surfactant crystals. When the water surfactant mixture reaches 35 °C, these crystals dissolve again. The enthalpy change of the transition increases upon increasing DDP content: more DDP molecules are solubilized at 35 °C.

In the enthalpogram of vesicles of EBP/DDP (4:1), the peak at 35 °C was not observed. We contend that the presence of an excess of EBP molecules inhibited the crystallization of DDP.

Both DSC and fluorescence depolarization show that increasing the proportion of DDP in vesicles composed of EBP lowers the  $T_m$  and makes the phase transition less cooperative and more complicated. The DSC measure-

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**Table 2. X-ray Diffraction Results**

sample	$d^a$ (0% RH)	$d^a$ (90% RH)
EBP	32.4, 29.9 <sup>b</sup>	32.6
EBP/DDP (4: 1)	32.4, 29.9 <sup>b</sup>	32.7
EBP/DDP (1:1)	29.8	33.2
EBP/DDP (1:4)	25.5 <sup>c</sup>	34.0
DDP	25.4 <sup>c</sup>	34.0

<sup>a</sup> In Å. <sup>b</sup> Small shadow peak. <sup>c</sup> No higher order reflection observed.

ments yield significantly lower  $T_m$ 's than the fluorescence depolarization measurements. The DSC measurements are apparently not fully in accord with the fluorescence depolarization measurements. This has been found before.<sup>35</sup> The vesicle solution used for DSC was of a much higher concentration than the solution used for the fluorescence depolarization measurements. This, and the fact that in DSC no probe is used, could account for the discrepancy between  $T_m$  values obtained by DSC and by fluorescence depolarization.

**X-ray Diffraction.** To determine whether the spacer segment of EBP was capable of spanning the membrane, low-angle X-ray diffraction was performed on dried vesicle preparations. The relative humidity (RH) in the measuring chamber was varied. At high relative humidity the amphiphile layers are hydrated. In Table 2 the results are summarized.

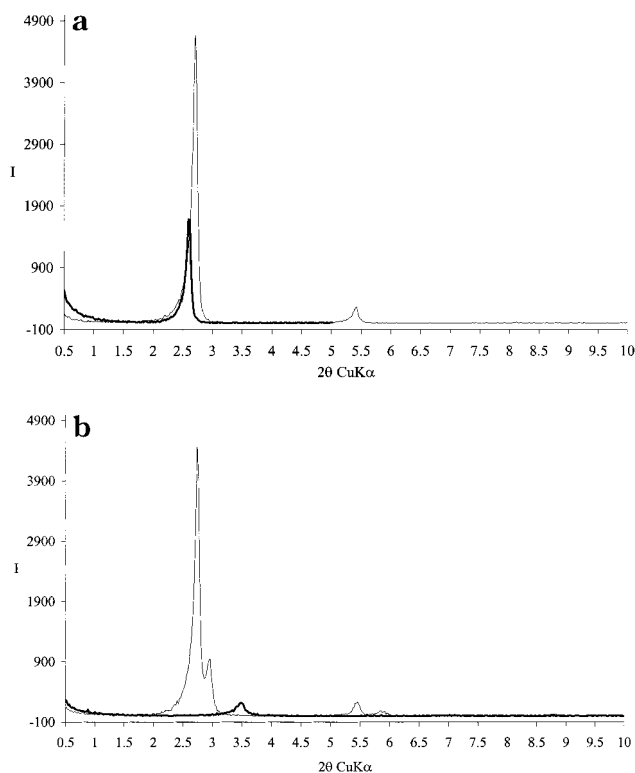
With the help of CPK models, the length of the bolaform molecule was estimated to be 29 Å. The width of a bilayer of DDP would be two times the length of the DDP molecule, estimated to be 15 Å, plus the distance between the terminal methyl groups of two DDP molecules forming a bilayer. The width of a bilayer of DDP molecules is therefore expected to be considerably larger than the width of a monolayer of EBP molecules.

The width of the hydrated bilayer (34.0 Å) is larger than that of the hydrated monolayer (32.6 Å) of the EBP molecules (Figure 5a). The width of the layer increases upon increasing DDP content. On the basis of these results we contend that the EBP molecule spans the vesicular membrane.

The difference in repeat spacing between the hydrated samples and the dehydrated samples is about 2.5 Å. This corresponds to the width of a layer of one water molecule between the amphiphile layers. The pure EBP layers and the EBP/DDP (4:1) layer undergo slow dehydration. Upon dehydration shadow peaks of low intensity appear, which are reflections of 29.9 Å, in accordance with the estimated width of the dehydrated amphiphile layer. Reflections corresponding to  $d = 32.3$  remain visible. The efficient packing of the EBP molecules in flat layers could cause the slow dehydration of the water molecules trapped between the layers.

The pure DDP bilayers and the layers composed of EBP/DDP (1:4) show complicated dehydration effects. When the relative humidity was reduced to 0%, only one reflection corresponding to 25.5 Å could be observed. This reflection was much less intense than the first-order reflection at 90% RH. The repeat spacing of 25.5 Å is not in accordance with the estimated width of a DDP bilayer. Apparently the dehydration distorts the lamellar packing (Figure 5b).

**Behavior upon Ca<sup>2+</sup> Addition.** Electron microscopy shows that after addition of Ca<sup>2+</sup> ions the EBP vesicles form aggregates at temperatures below  $T_c$ . However at  $T > T_c$  no aggregates are observed. Immediately upon Ca<sup>2+</sup>



**Figure 5.** XRD of dried samples (a) of pure EBP (lighter line) and of pure DDP (heavier line), RH 90%, and (b) of pure EBP (lighter line) and of pure DDP (heavier line), RH 0%.

addition tubule-like structures are formed. Upon addition of EDTA these tubules neither dissolve again nor revert into vesicles (Figure 6). These tubules have been observed before when the fusogenic behavior of di-*n*-dodecylphosphate was studied, and they turned out to be anhydrous calcium didodecylphosphate crystals. However, the calcium dialkyl phosphate crystals did revert to vesicles upon addition of EDTA. Other dialkylphosphates show the same behavior.<sup>36,37</sup> Other lipids show also morphological changes upon Ca<sup>2+</sup> addition. Phosphatidylserine vesicles form spirally folded multilamellar cylinders upon Ca<sup>2+</sup> addition; after addition of EDTA the cylinders turned into large unilamellar vesicles.<sup>38</sup> The rate of formation of calcium di-*n*-dodecyl phosphate crystals was much lower than the rate of formation of the bolaform calcium phosphate tubules observed here. A solution of the bolaform vesicles, which was initially clear, turned turbid immediately after Ca<sup>2+</sup> addition, and samples taken after less than a minute of incubation time showed the tubules.

Vesicles composed of EBP/DDP 1:1 and 4:1 show a different behavior (Figure 7). No tubules were observed after addition of Ca<sup>2+</sup>, and after addition of EDTA the aggregates obtained upon Ca<sup>2+</sup> revert into separate vesicles. These vesicles are larger, typically 200–600 nm, than the vesicles before addition of EDTA and Ca<sup>2+</sup> (typically 10–50 nm), so it seems that Ca<sup>2+</sup>-induced fusion has taken place.

Attempts to follow fusion by a lipid mixing assay failed. Two different assays were employed (the RET assay<sup>39</sup> and

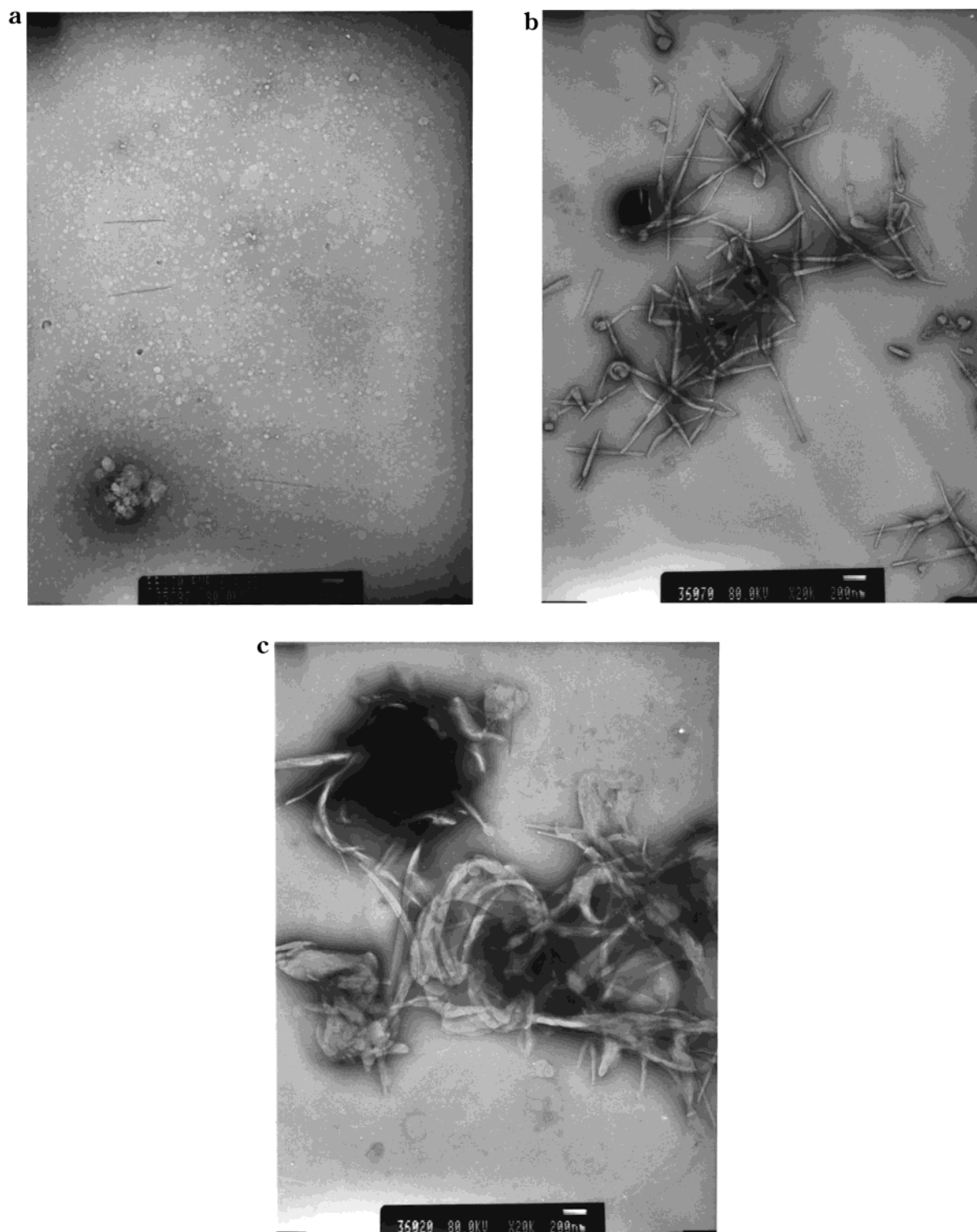
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**Figure 6.** Electron micrographs of (a) EBP vesicles (b) after addition of  $\text{Ca}^{2+}$  and (c) after addition of EDTA.

the Rhodamine 18 assay<sup>40</sup>). Probably the probe does not blend homogeneously with the amphiphiles in the bilayer.  $\text{Ca}^{2+}$  also induces phase separation. Upon phase separation the local concentration of a probe increases and an increase in self-quenching occurs. The rate of phase separation (half-life ca. 1 min) in phosphatidylserine vesicles is smaller than the rate of fusion (half-life < 5 s).<sup>41</sup> It could be that in EBP and EBP/DDP vesicles, the rate

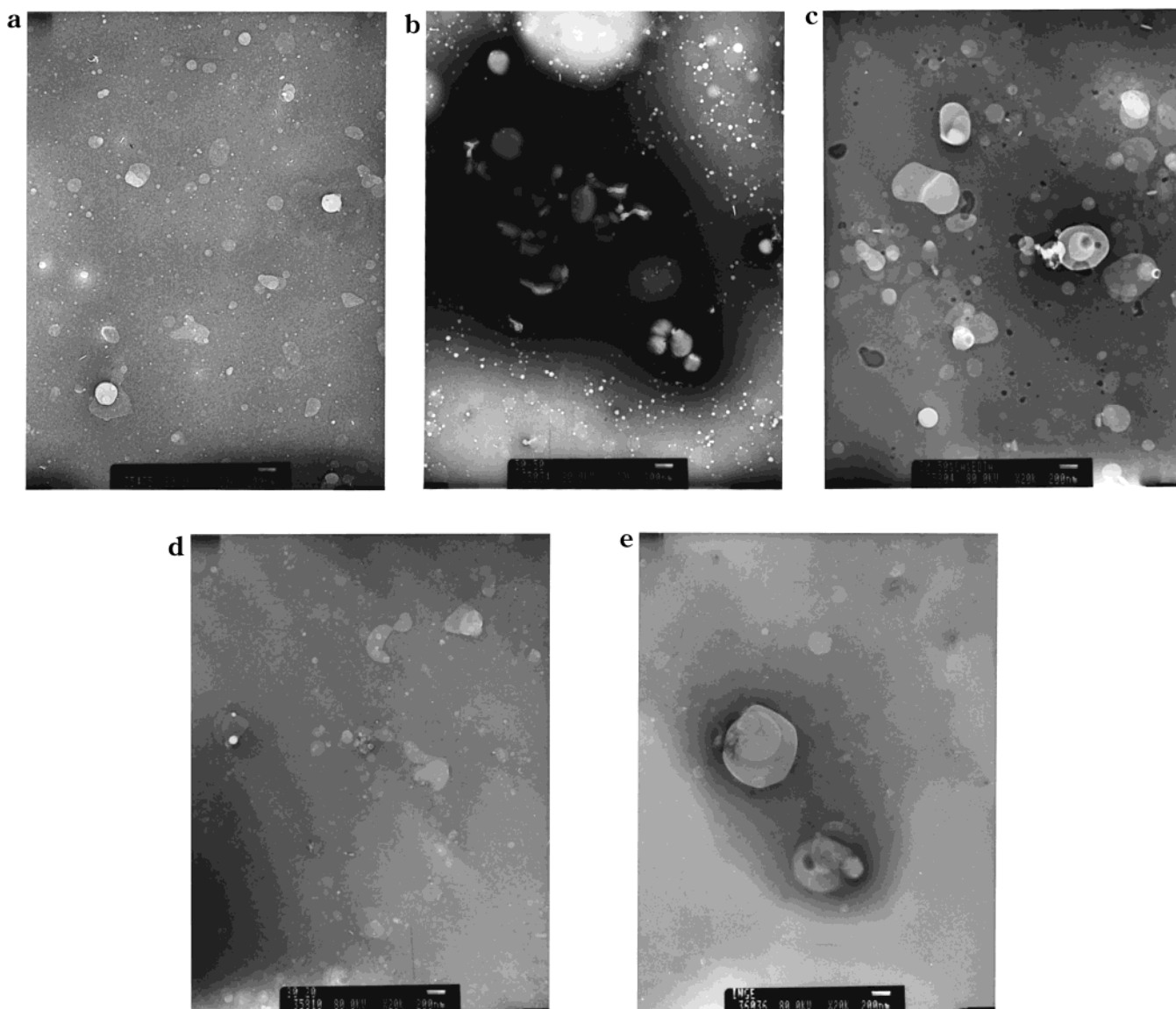
of phase separation is much faster than the fusion rate. In other words, calcium crystals are formed at a higher rate than fusion occurs.

Attempts to encapsulate carboxyfluorescein into vesicles of pure EBP failed, due to instability of the vesicles in high ionic strength solutions and, consequently, no contents mixing assays can be employed.

**Conclusions.** Mixtures of different ratios of sodium di-*n*-decylphosphates and sodium di-*n*-decyl  $\alpha,\omega$ -eicosanyl bisphosphate form vesicles. By variation of the composition of the vesicles the properties of the vesicles are influenced

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**Figure 7.** (a) Electron micrographs of EBP/DDP vesicles (1:1) (b) after addition of  $\text{Ca}^{2+}$  and (c) after addition of EDTA. (d) EBP/DDP vesicles (4:1) (e) after addition of calcium and EDTA.

significantly. The colloidal stability decreased upon increasing DDP content. The main phase transition temperature decreased going from pure EBP (31.7 °C) to EBP/DDP (4:1) (27.9 °C).

X-ray diffraction studies show that the bolaform amphiphile is membrane spanning.

Upon  $\text{Ca}^{2+}$  addition the EBP vesicles form tubules, which are presumably anhydrous calcium crystals of di-*n*-decyl  $\alpha,\omega$ -eicosanyl bisphosphate. To allow fusion of vesicles of charged bola-amphiphile vesicles, it is necessary to add nonbolaform amphiphiles.

### Experimental Section

**Materials.** *N*-Decyl phosphate was prepared according to a standard literature procedure.<sup>42</sup> Di-*n*-decyl phosphate was prepared as described previously.<sup>43</sup> 11-Bromoundecanoic acid was purchased from Aldrich, sodium acetate from Merck, and *N*-[2-hydroxyethyl]piperazine-*N*-[2-ethanesulfonic acid] (HEPES) from Sigma Chemical. Chemicals were used as received. All water used in the experiments was distilled twice in an all-quartz

distillation apparatus. In some experiments vesicles were prepared in a 5 mM/ 5 mM HEPES/NaAc buffer of pH 7.4.

NMR spectra were recorded on a Varian Gemini 200 or a Varian VXR-300 instrument, using the chemical shifts of the solvents as internal standards. Melting points were determined on a Mettler FP1 melting point apparatus equipped with an Ernst Leitz Wetlar microscope 411657. Mass spectra were recorded using an AEI-MS9 mass spectrometer.

**1,20-Dibromoeicosane.** This compound was synthesized from 11-bromoundecanoic acid using the Kolbe electrolysis.<sup>44</sup> Ten grams (0.038 mol) of 11-bromoundecanoic acid were dissolved in 80 mL of methanol. Then 0.09 g (0.004 mol) of sodium metal was added. After the metal was dissolved, a current of 0.7 A was applied, using platinum electrodes, until the methanol was slightly basic to pH indicator paper. The precipitate was filtered off and recrystallized from methanol. The platelet crystals melted at 68 °C (lit. 66.5–67).<sup>44</sup> Yield 55%. <sup>1</sup>H-NMR (200MHz,  $\text{CDCl}_3$ ):  $\delta$  3.40 (t, 6.8 Hz, 4H); 1.84 (m, 4H); 1.2 (m, 32H).

**Disodium Di-*n*-decyl  $\alpha,\omega$ -Eicosanyl Bisphosphate.** The bolaform amphiphile was synthesized analogous to the preparation of the bolaform amphiphiles described previously,<sup>45</sup>

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following the method described by Bauman.<sup>46</sup> In short, 4 equiv of *n*-decyl phosphate was dissolved in a 25% methanolic N(CH<sub>3</sub>)<sub>4</sub>-OH solution (8 equiv). The solution was stirred for an hour. After removal of the methanol in vacuo, 30 mL of acetonitrile was added to the oily residue and to the stirred solution 1.1 g (2.5 mmol) of dibromoeicosane was added. After the mixture was refluxed for 6 days, the acetonitrile was removed in vacuo. The residue was dissolved in water and acidified to pH 0.5. The precipitate was collected on a Büchner funnel, suspended in ethanol 96%, filtered, and then suspended in ether, and the suspension stirred for an hour to remove the excess of *n*-decyl phosphate. The precipitate was collected and recrystallized from absolute ethanol. Yield: 82.7 % Mp: 103.5–104.5 °C. <sup>1</sup>H-NMR: 4.02 (q, 6.6 Hz, 8H), 1.68, (m, 8H), 1.27, (m, 62H), 0.88, (m, 6H). <sup>31</sup>P-NMR: 1.34 ppm, s. Anal. Calcd for C<sub>40</sub>H<sub>84</sub>P<sub>2</sub>O<sub>8</sub>·1/2H<sub>2</sub>O: H, 11.22; C, 62.86; P, 8.11. Found: H, 11.01; C, 63.08; P, 8.05.

Prior to the experiments the phosphate was converted into its disodium salts by refluxing in absolute ethanol and neutralizing the solution with an ethanolic NaOEt solution. After removal of the ethanol in vacuo, the white solid was crystallized from absolute ethanol.

**Vesicle Preparation.** Vesicles were prepared by removing the solvent from the desired amount of stock solution of the amphiphile in methanol (EBP) or chloroform (DDP). The resulting thin amphiphile layer is hydrated with HEPES/NaAc buffer or double-distilled water and subsequently sonicated with a probe type sonicator for a few minutes at 50 °C. For DSC and fluorescence depolarization measurements the vesicles were prepared by dissolving the appropriate amount of amphiphile in water and stirring the solution at  $T > 50$  °C.

**Fluorescence Polarization.** The measurements were performed with a SLM Aminco SPF 500C spectrofluorometer equipped with a thermostated cell holder. 1,6-Diphenyl-*trans,trans,trans*-1,3,5-hexatriene (DPH) (concentration =  $5 \times 10^{-8}$  M) was excited at 360 nm. The emission wavelength was 428 nm (bandpass 5 nm). The fluorescence polarization was calculated from the intensities of the emitted light parallel and perpendicular to the direction of the excitation radiation using  $P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$ . The amphiphile concentration was  $5 \times 10^{-5}$  M. Measurements involved heating scans with 3 °C intervals. The samples were allowed to equilibrate for 10 minutes after each temperature increase. Reported values for  $P$  are average values of six independent measurements.<sup>47</sup>

**Differential Scanning Calorimetry (DSC).** DSC measurements were performed at the University of Leicester with a

differential scanning microcalorimeter (MicroCal Ltd., USA) as previously described.<sup>48,49</sup> A  $5.0 \times 10^{-3}$  M solution of amphiphile in water was heated to 60 °C and stirred for 30 minutes. After cooling to room temperature and degassing, the solution was injected into the sample cell of the microcalorimeter and scanned by heating immediately after cooling to low temperature. In addition, scans have been made 11 h after equilibration below the phase transition temperature. Analyses of the scans were executed using the ORIGIN software.

**Electron Microscopy.** Samples were prepared by placing a 1 mM solution on an air glow-discharged carbon-coated Formvar grid. This was followed after 20 s by staining with a 1% phosphotungstic acid solution. The samples were examined in a JEOL electron microscope operating at 80 kV.

**XRD Measurements.** Samples were prepared by putting a few drops of the amphiphile solution (ca. 5 mM) in bidistilled water on a Si single-crystal wafer, cut along the (501) plane, followed by lyophilization. Measurements were performed on an optimized home-built (NIOZ, Texel) high-accuracy  $\theta$ – $\theta$  diffractometer equipped with a CuLFF tube, a variable divergence and antiscatter slit, and an energy dispersive Si/Li detector (Kevex), which enables a high peak to background ratio.<sup>50</sup> The relative humidity of the measuring chamber was controlled. The samples were allowed to equilibrate for 10 min after each adjustment of the relative humidity.

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